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EXPERIMENTAL THERAPEUTICS

Insecticidal Activity of Doxycycline against the Common Bedbug

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ABSTRACT There is an ongoing need for safe and effective anti-bedbug compounds. Here, we tested the toxicity of three antimicrobial agents against bedbugs when administered orally. We reveal that doxycycline has direct insecticidal activity at 250 μg/ml (0.025%) that is particularly strong against immature bedbugs and appears to be independent of antimicrobial activity. Future studies to determine the mechanisms behind this property could be useful for the development of orally active insecticides or anti-bedbug therapeutics.

KEYWORDS *Cimex*, bedbug, doxycycline, insecticidal, insecticide, toxicity

The common bedbug, *Cimex lectularius*, is a blood-feeding insect pest of economic and public health importance. Because bedbugs live in close association with their human hosts, individuals suffering from infestations frequently experience psychological disturbances such as anxiety and insomnia (1). In addition, the bedbug feeding process can trigger allergic reactions, such as urticaria and bullous lesions, which can lead to secondary infections (2). Rare cases of anaphylaxis due to bedbug bites have also been reported (2). For these reasons, significant time and resources are spent yearly on treatments for bedbug infestations (3). However, today there are growing concerns about the development of resistance against commonly used chemical control methods in populations of bedbugs across the globe (4–6).

Targeting commensal bacteria is one potential alternative strategy for control of a broad range of arthropod pests. Many insect species have evolved mutualistic relationships with bacteria and depend on these for various aspects of their physiology (7). As a result, eliminating bacterial symbionts has long been known to have adverse effects on the insect's life course (8–10). Antibacterial treatments have even been shown to cause mortality through indirect routes. For instance, in the tsetse fly, death coinciding with an accumulation of dead bacteria occurs within days of feeding on rabbits dosed with tetracycline (11). Further, the production of detoxification enzymes by symbiotic bacteria can aid host insects in the degradation of pesticides, suggesting that antibacterial treatment may enhance the activity of other insecticidal compounds (12–15). Indeed, in human lice, daily consumption of 10 to 50 μg/ml (0.001% to 0.005%) of doxycycline in blood causes delayed, indirect mortality by killing the louse endosymbiont, but it also has a synergistic effect on the antiparasitic agent ivermectin (16, 17).

Bedbugs rely on proteobacteria, particularly *Wolbachia*, that reside in a specialized organ called the bacteriome for the synthesis of B vitamins. Reducing the titer of these bacteria through continuous rearing on blood containing 10 μg/ml (0.001%) rifampin stunts bedbug development and reproduction (18, 19). Thus, we initially designed a study to examine whether different doses of rifampin or other antimicrobials could elicit more pronounced adverse effects on the life history of bedbugs. During the course of that study, we serendipitously observed that bedbugs fed higher doses of doxycycline exhibited rapid and pronounced mortality, which was an unexpected

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TABLE 1 Combined 7-day survival of adult and immature *C. lectularius* fed antimicrobials

Stage	No. surviving/total (%) fed[a]:			
	Penicillin[b]	Rifampin[b]	Doxycycline[c]	Blood
Nymph	34/36 (94.4)	38/40 (95)	1/55 (1.8)*	41/44 (93.2)
Adult	32/32 (100)	34/34 (100)	12/34 (35.3)*	40/42 (95.2)

[a]Antimicrobials were administered at 250 μg/ml. *, statistically significant difference relative to the blood-only control ($P < 0.0001$).
[b]Two replicates of 15 to 20 insects per group.
[c]Three replicates of 10 to 23 insects per group.

finding unique to this antimicrobial. Here, we report the results of experiments in which adults and first-instar nymphs of *C. lectularius* were fed a single blood meal spiked with 250 μg/ml (0.025%) of either doxycycline, rifampin, or penicillin through an artificial membrane feeder. Further, gross imaging of bedbugs that died from doxycycline ingestion was carried out to explore possible causes of mortality.

The Cincinnati field strain of *C. lectularius* was used in all experiments. This strain is derived from insects collected by technicians from Sierra Research Laboratories (Modesto, CA) in Cincinnati, OH, in 2007. The insects were maintained in plastic enclosures with cardboard harborages under ambient conditions (~25°C and 40% to 45% relative humidity) on a 12:12 photoperiod. The colonies were typically fed commercially purchased, mechanically defibrinated rabbit blood (Hemostat, Dixon, CA) once per week using an artificial membrane system (Apex Bait Technologies, Inc., Santa Clara, CA). To prepare the experimental blood meals, doxycycline hyclate (Sigma-Aldrich, St. Louis, MO) was dissolved in water and diluted 1:100 in defibrinated rabbit blood (Hemostat) to a final concentration of 250 μg/ml (0.025%). Penicillin sodium (Sigma-Aldrich) was similarly dissolved in water and diluted 1:100 in rabbit blood to a final concentration of 250 μg/ml (0.025%), while rifampin (Sigma-Aldrich) was dissolved in methanol and diluted 1:100 in rabbit blood to a final concentration of 250 μg/ml (0.025%). An artificial membrane device (Apex Bait Technologies, Inc.) was used to provide the blood to separate groups of adults or first-instar nymphs until most insects were fully engorged. Insects that did not feed were removed from experimental enclosures and discarded. For doxycycline treatment, data were collected from 3 replicates of 10 to 23 insects per group. For penicillin and rifampin treatments, data were collected from 2 replicates of 15 to 20 insects per group. Mortality was recorded for a period of 7 days after feeding, and insects fed rabbit blood with no additives served as controls. Differences in survival at 7 days postfeeding were analyzed using the chi-square Fisher's exact test in GraphPad (GraphPad Software, San Diego, CA).

Survival in control groups fed only defibrinated rabbit blood was >90% on average at all time points examined. We found that neither rifampin nor penicillin had any effect on bedbug survival, as the 7-day survival of adults fed either antimicrobial was 100% (Table 1). For nymphs fed the same compounds, 7-day survival was greater than 90%. On the other hand, doxycycline caused significant mortality in both nymphs and adults (Table 1). Only 1.8% of nymphs ($P < 0.0001$) and 35.3% of adults ($P < 0.0001$) fed doxycycline survived to day 7 postfeeding, a statistically significant decrease relative to blood-only controls. The effect of doxycycline on adult bedbugs was gradual (Fig. 1A), as survival steadily declined between 1 day and 7 days postfeeding with a median lethal time (LT_{50}) of 3.48 days based on regression analysis ($r^2 = 0.943$). Toxicity to nymphs, meanwhile, appeared to be rapid (Fig. 1B), as the bulk of mortality occurred within the first day after ingesting doxycycline ($LT_{50} < 1$ day). However, when we fed doxycycline at a concentration of 5 μg/ml (0.0005%), 100% survival was observed in both nymphs and adults ($n = 3$ replicates, 41 to 57 total insects). This was despite the fact that this concentration remains highly effective against symbiotic bacteria (20).

Bedbugs that had died from doxycycline ingestion were morphologically examined 24 h after feeding. The insects were visualized in a dark room on a Leica M165FC stereomicroscope (Leica, Weltzlar, Germany) and photographed with a DFC 310 FX camera (Leica) using Leica Application Suite software. Several interesting features were

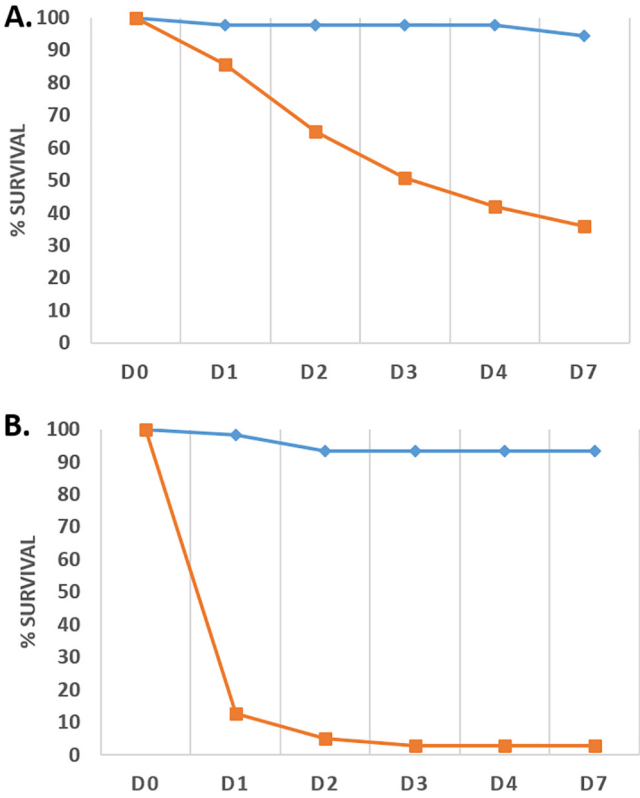

FIG 1 Oral toxicity of doxycycline to adult (A) and first-instar (B) *C. lectularius*. Insects were fed untreated blood (blue) or 250 μg/ml doxycycline (orange) as described in the text, and survival was measured over the course of 7 days. Data points represent the mean survival from 3 replicates of 10 to 23 insects per group after unfed individuals were removed from the experiments.

evident (Fig. 2). The insects appeared to have died prior to any digestion of the blood meal, as the bodies were swollen with red fluid (Fig. 2A and B). In contrast, the partially digested blood meal in healthy individuals typically appears small and dark within 24 h of feeding (Fig. 2C). Closer inspection revealed that the fluid buildup was in the body

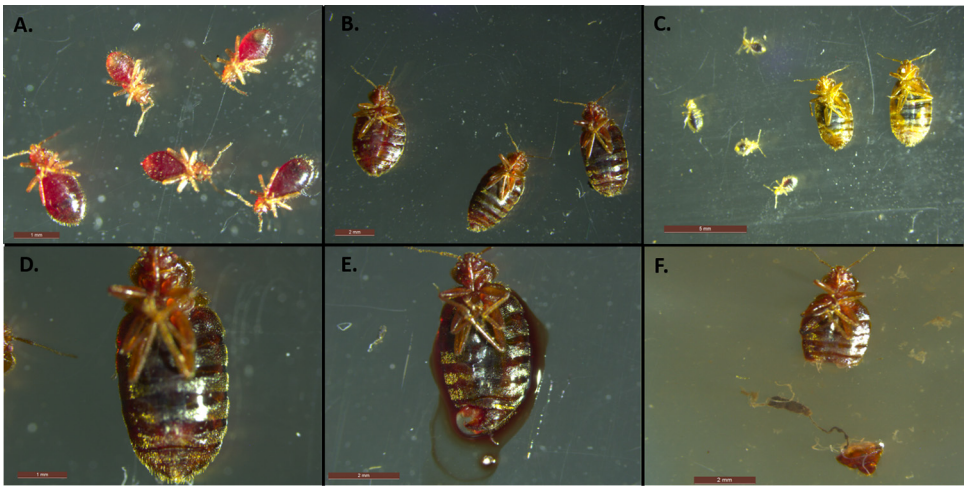

FIG 2 Dead bedbugs 24 h after ingestion of 250 μg/ml doxycycline. (A) Nymphs; (B) adults; (C) live controls fed blood only. (D) Magnified view demonstrating swelling and red coloration throughout the abdomen. (E) Removal of the posterior tip of the abdomen resulted in release of a large amount of red fluid from the hemocoele. (F) Dissection of the gut after washing off fluid revealed an intact posterior midgut and hindgut. Images are representative of results seen in each replicate.

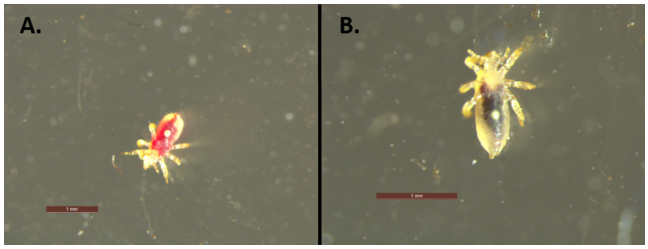

FIG 3 Human body lice fed 250 μg/ml doxycycline. (A) First-instar nymph immediately after engorging. (B) First-instar nymph 24 h after ingestion, demonstrating digestion of the blood meal. Images are representative of multiple individuals.

cavity but outside the gut (Fig. 2D), and gentle removal of the posterior tip of the abdomen led to the release of a large amount of fluid from the hemocoele (Fig. 2E). Nonetheless, the midgut and hindgut appeared intact (i.e., unruptured) at the gross level upon dissection of the digestive tract (Fig. 2F), suggesting that major structural damage to these sections of the gut was not a major contributor to mortality.

To determine if the effects of doxycycline were specific to bedbugs, we next administered 250 μg/ml (0.025%) doxycycline to laboratory-reared first-instar nymphs of the human body louse, *Pediculus humanus humanus*, using the same feeding procedure that was used for bedbugs. In this preliminary experiment, all immature body lice fed doxycycline in rabbit blood were able to process the blood meal, and no abnormal fluid buildup was noted (Fig. 3).

Our results demonstrate that ingestion of a single dose of doxycycline can be toxic to bedbugs when administered at an appropriate concentration. Doxycycline is a tetracycline antimicrobial agent that functions primarily by inhibiting ribosome function to prevent protein synthesis, and it has a broad spectrum of activity that includes both Gram-positive and Gram-negative bacteria as well as protozoan parasites (21). The lack of toxicity observed in bedbugs after feeding on other antimicrobials at the same concentration may be due to host detoxification mechanisms that result in reduced absorption and/or metabolism of these compounds. Alternatively, the antimicrobials may have differential activity against various bedbug symbionts. For example, penicillin is not effective against *Wolbachia*, although rifampin and doxycycline are (20, 22). However, given the high rate of death documented in nymphs, the morphological phenotypes apparent after ingestion of doxycycline, and the lack of short-term mortality in body lice, the most plausible explanation is that the effect we observed is not due to killing of bedbug symbionts but rather a direct action of doxycycline on bedbugs. This is a surprising and significant new finding that should be confirmed in future studies by measuring bacterial viability via PCR or fluorescence microscopy following antibiotic treatment, alongside more detailed examinations of damage to the host. Moreover, it would be interesting to examine dose responses across bedbug strains with various susceptibilities to insecticides.

The discovery that doxycycline is toxic when ingested by bedbugs may potentially be useful for controlling infestations in several ways. It has been suggested that afflicted humans could be treated with anti-parasitic drugs such as ivermectin or moxidectin to help mitigate infestations in combination with other pest management approaches (23–26). Although doxycycline could be a milder pharmaceutical alternative to antiparasitic drugs, the peak plasma concentration in humans treated with doxycycline is 50- to 100-fold lower than the concentration we show to have insecticidal activity (23, 27, 28). When we tested a concentration in this range (i.e., 5 μg/ml [0.0005%]), no mortality was observed. Therefore, it is unlikely that doxycycline itself will be clinically useful against bedbugs. Nonetheless, concentrations of doxycycline in this range have been previously shown to enhance the effects of antiparasitic agents against bedbugs, and the activity of other tetracycline antimicrobials may merit further investigation (23). In considering this pharmacological approach, it is important to note that even mild antimicrobials could have undesirable side effects.

On the other hand, we propose that understanding the mechanism(s) by which doxycycline exerts its toxicity on bedbugs has more substantial implications and is of interest. In addition to their antimicrobial properties, several tetracycline antimicrobials, including doxycycline, are known to have aquaretic effects on mammals and amphibians by modulating the expression of aquaporin genes in kidney cells (29). That is, the drugs can increase water excretion and electrolyte retention simultaneously. Intriguingly, bedbugs harbor several aquaporin-like genes, and RNA interference silencing of these genes reduces the insects' ability to excrete water following a blood meal (30). Perhaps doxycycline ingestion leads to a dysregulation of aquaporin activity that ultimately results in fluid leakage into the hemocoele, as we observed. Deploying doxycycline or other tetracycline antimicrobials into the environment to directly target bedbugs is unadvisable due to concerns that this could contribute to the rise of antibiotic-resistant bacteria. However, elucidating the mode(s) of action of doxycycline may provide leads for the development and/or optimization of orally active anti-bedbug compounds that could have application in emerging control technologies such as liquid baits.

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